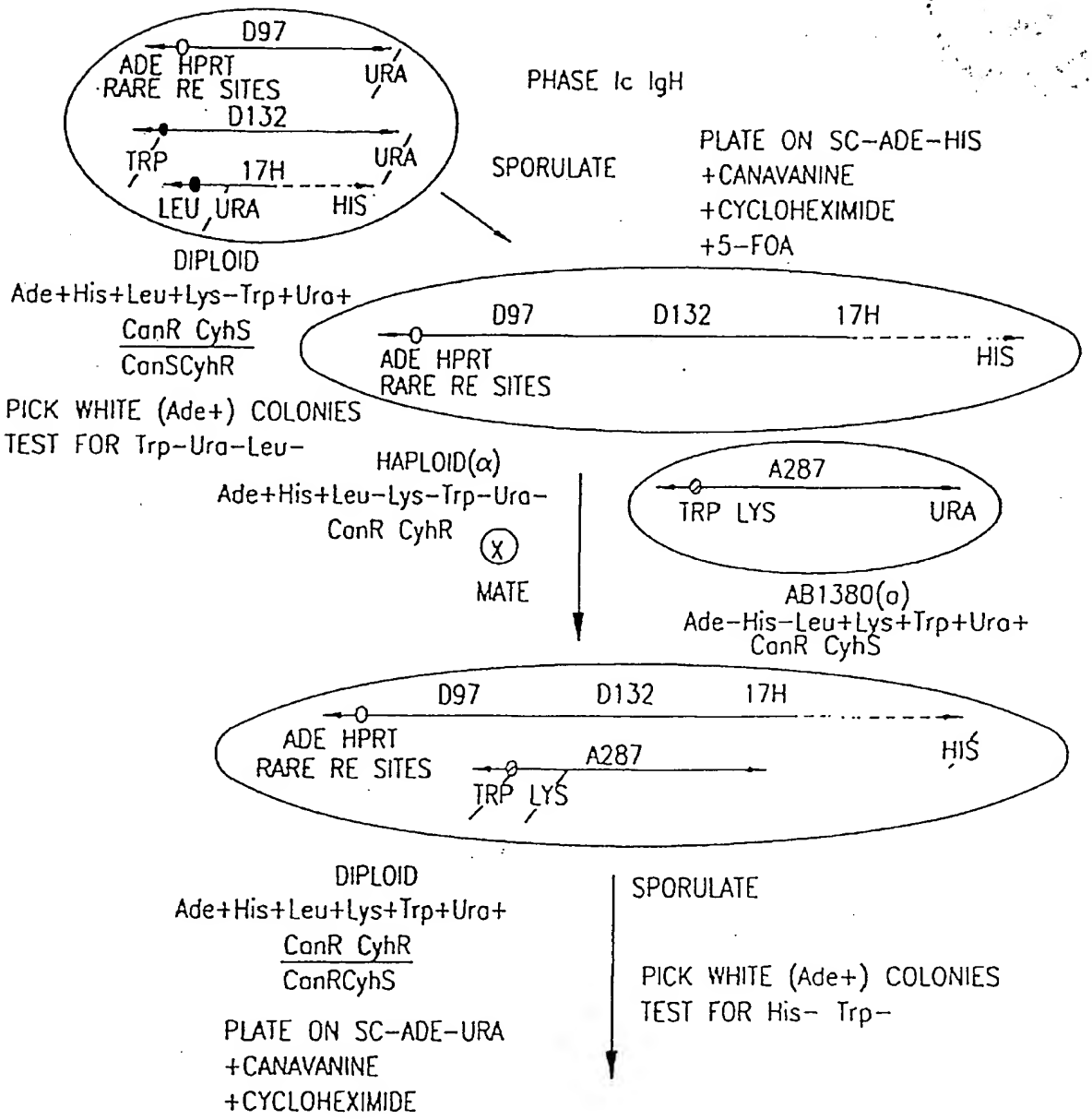
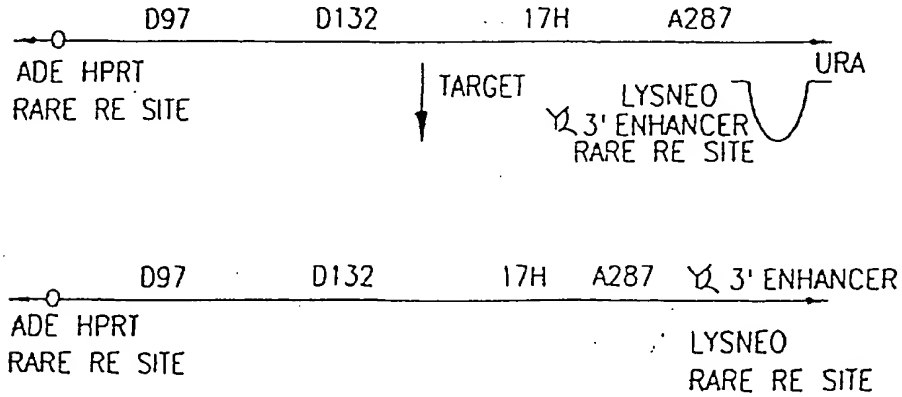

D97
ADE HPRT
RARE RE SITES
URA
D132
TRP
17H
URA
LEU
URA
HIS
PHASE Ic IgH
SPORULATE
PLATE ON SC-ADE-HIS
+CANAVANINE
+CYCLOHEXIMIDE
+5-FOA
DIPLOID
Ade+His+Leu+Lys-Trp+Ura+
ConR CyhS
ConSCyhR
PICK WHITE (Ade+) COLONIES
TEST FOR Trp-Ura-Leu-
D97 D132 17H
ADE HPRT
RARE RE SITES
HIS
HAPLOID(α)
Ade+His+Leu-Lys-Trp-Ura-
ConR CyhR
X
MATE
A287
TRP LYS
URA
AB1380(a)
Ade-His-Leu+Lys+Trp+Ura+
ConR CyhS
D97 D132 17H
ADE HPRT
RARE RE SITES
A287
HIS
TRP LYS
DIPLOID
Ade+His+Leu+Lys+Trp+Ura+
ConR CyhR
ConRCyhS
SPORULATE
PICK WHITE (Ade+) COLONIES
TEST FOR His- Trp-
PLATE ON SC-ADE-URA
+CANAVANINE
+CYCLOHEXIMIDE

FIG.1


D97 D132 17H A287
ADE HPRT
RARE RE SITE
URA
TARGET
LYSNEO
3' ENHANCER
RARE RE SITE
D97 D132 17H A287 3' ENHANCER
ADE HPRT
RARE RE SITE
LYSNEO
RARE RE SITE

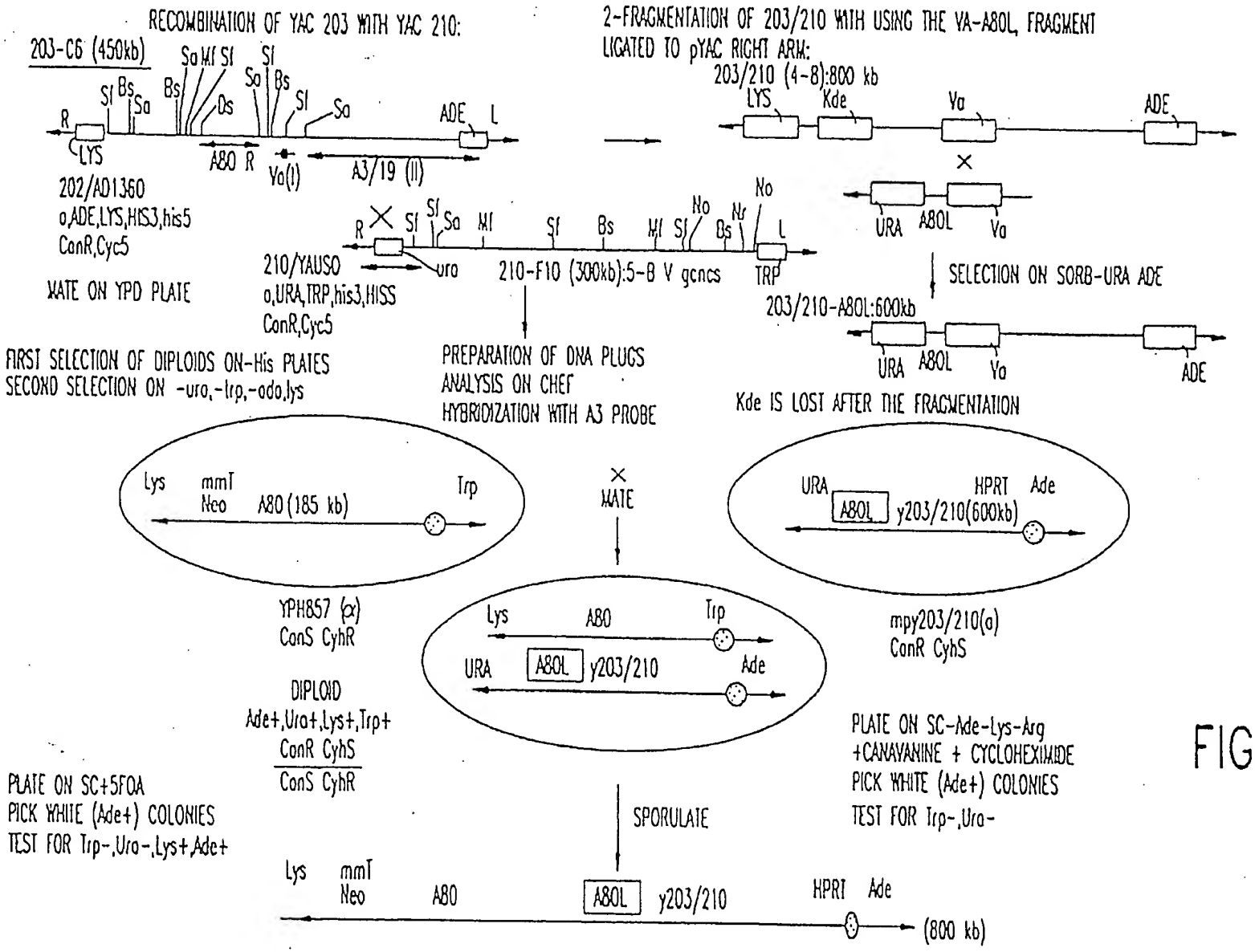


FIG.2

08/724752

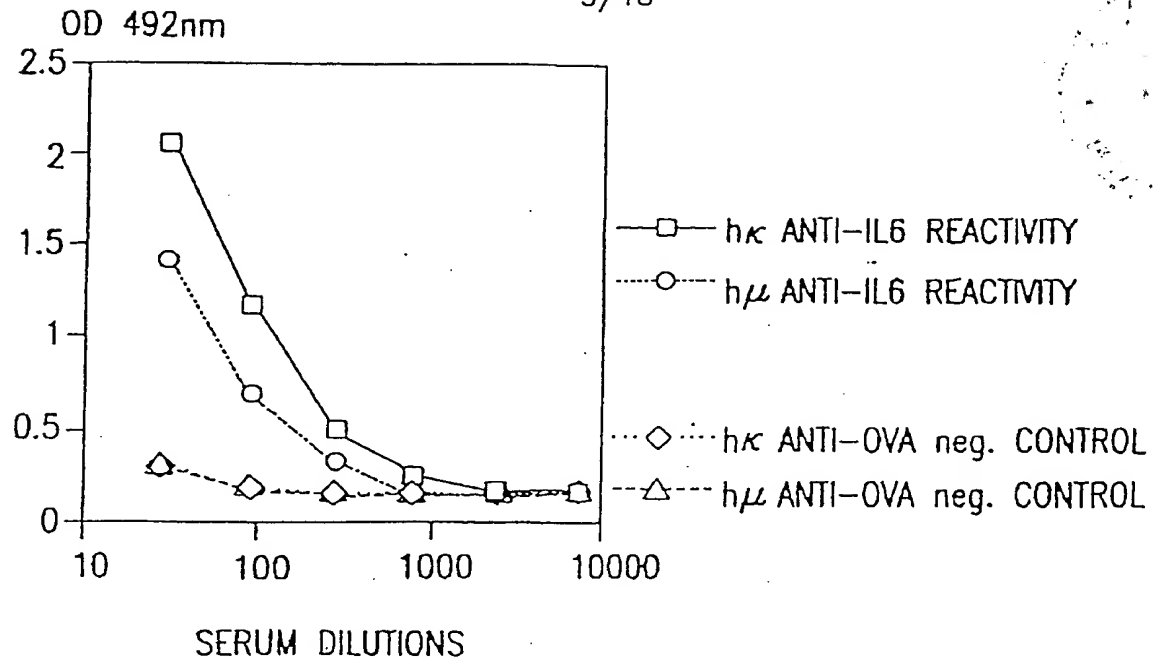

OD 492nm
2.5
2
1.5
1
0.5
0
10
100
1000
10000
SERUM DILUTIONS
hκ ANTI-IL6 REACTIVITY
hμ ANTI-IL6 REACTIVITY
hκ ANTI-OVA neg. CONTROL
hμ ANTI-OVA neg. CONTROL

FIG.3

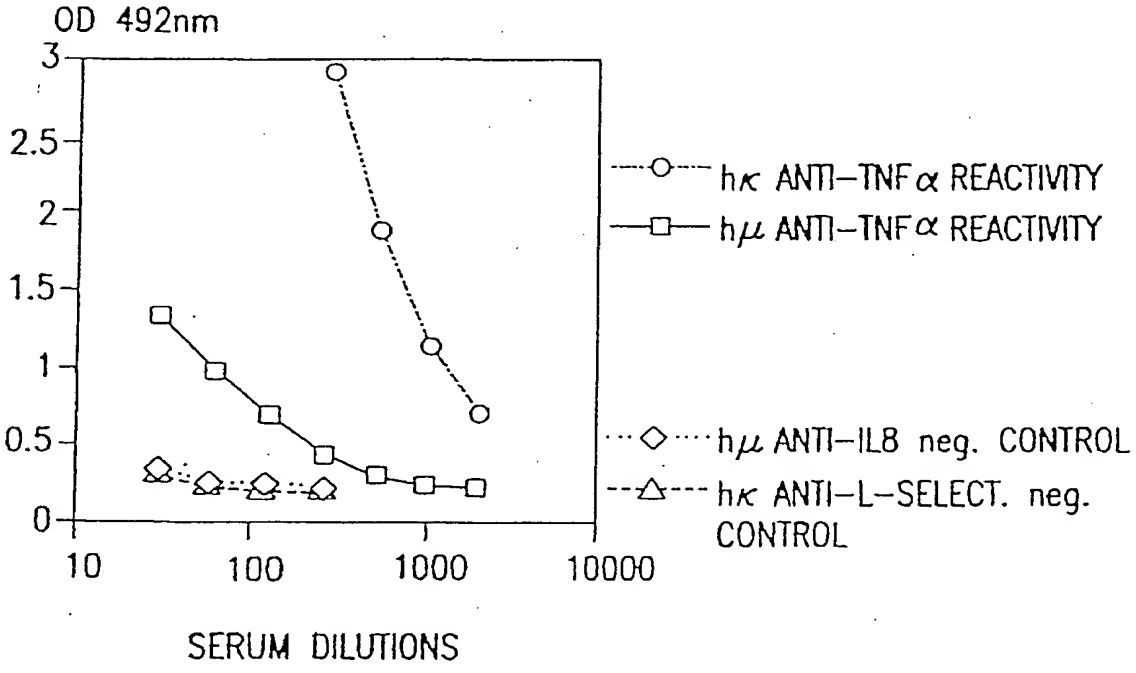

OD 492nm
3
2.5
2
1.5
1
0.5
0
10
100
1000
10000
SERUM DILUTIONS
hκ ANTI-TNFα REACTIVITY
hμ ANTI-TNFα REACTIVITY
hμ ANTI-IL8 neg. CONTROL
hκ ANTI-L-SELECT. neg. CONTROL

FIG.4

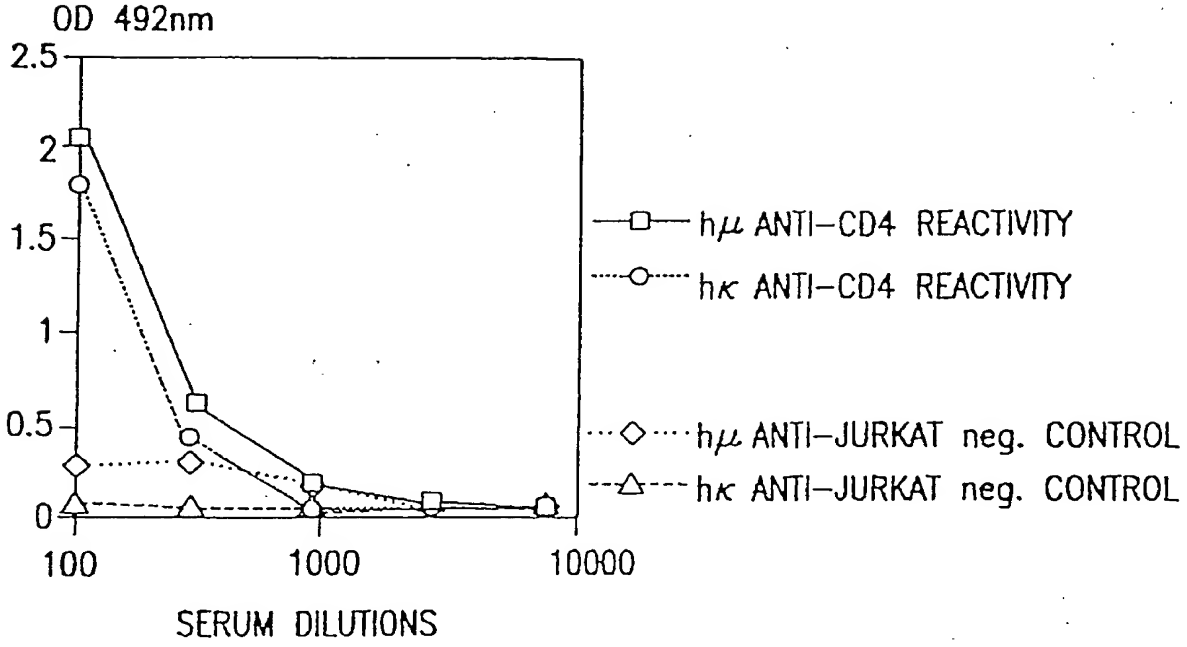

OD 492nm
2.5
2
1.5
1
0.5
0
100
1000
10000
SERUM DILUTIONS
hμ ANTI-CD4 REACTIVITY
hκ ANTI-CD4 REACTIVITY
hμ ANTI-JURKAT neg. CONTROL
hκ ANTI-JURKAT neg. CONTROL

FIG.5

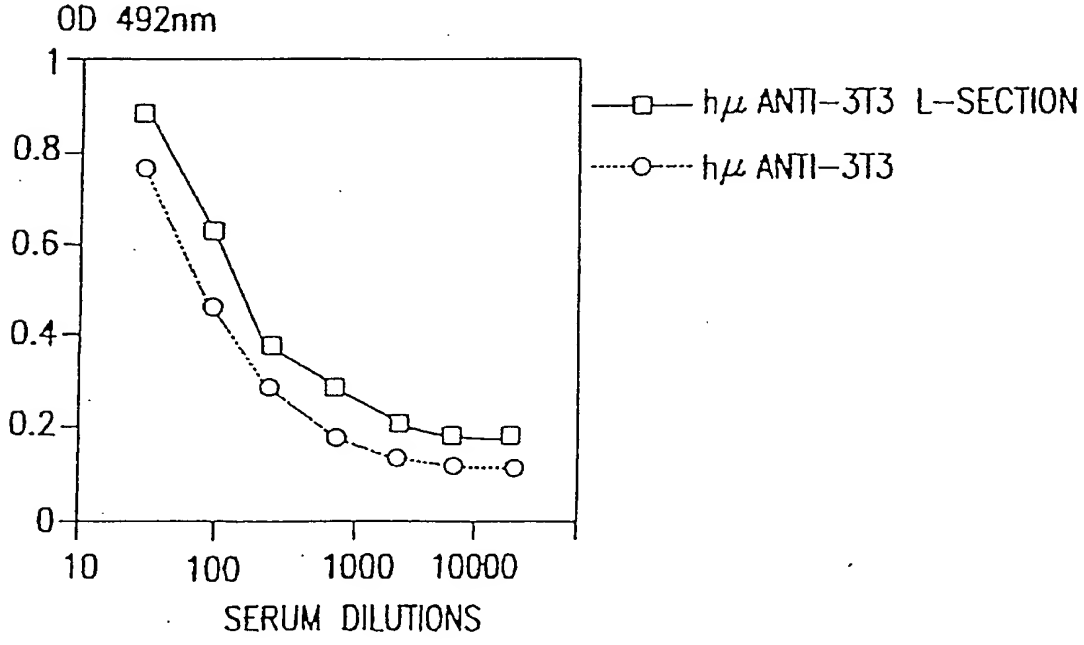

OD 492nm
1
0.8
0.6
0.4
0.2
0
10
100
1000
10000
SERUM DILUTIONS
hμ ANTI-3T3 L-SECTION
hμ ANTI-3T3

FIG.6

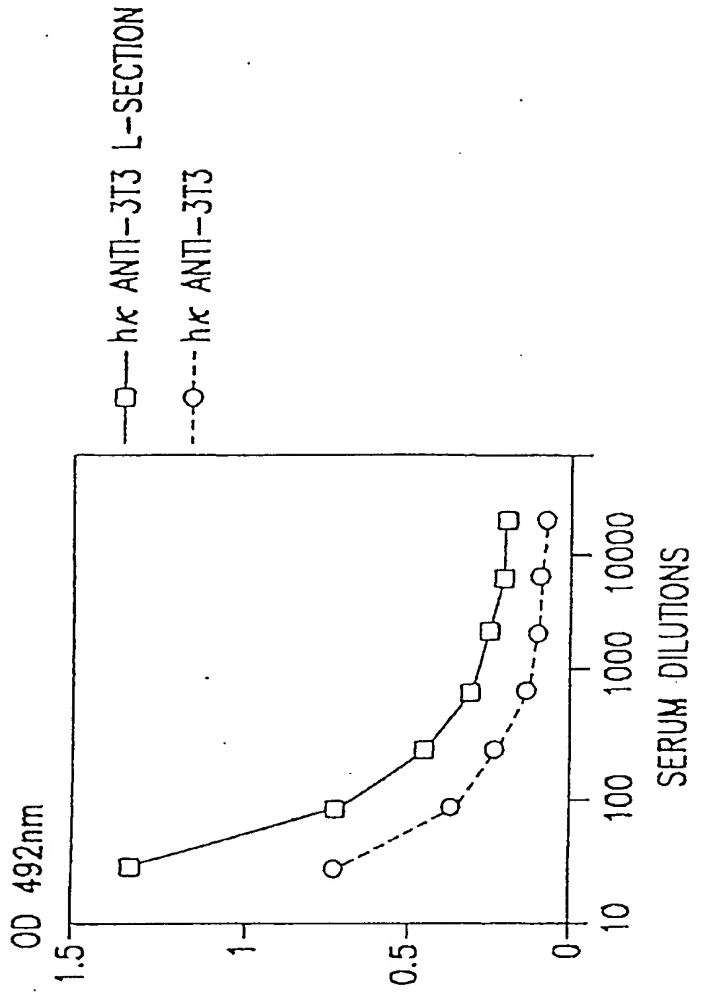

hκ ANTI-3T3 L-SECTION
hκ ANTI-3T3
OD 492nm
1.5
1
0.5
0
10
100
1000
10000
SERUM DILUTIONS

FIG.7

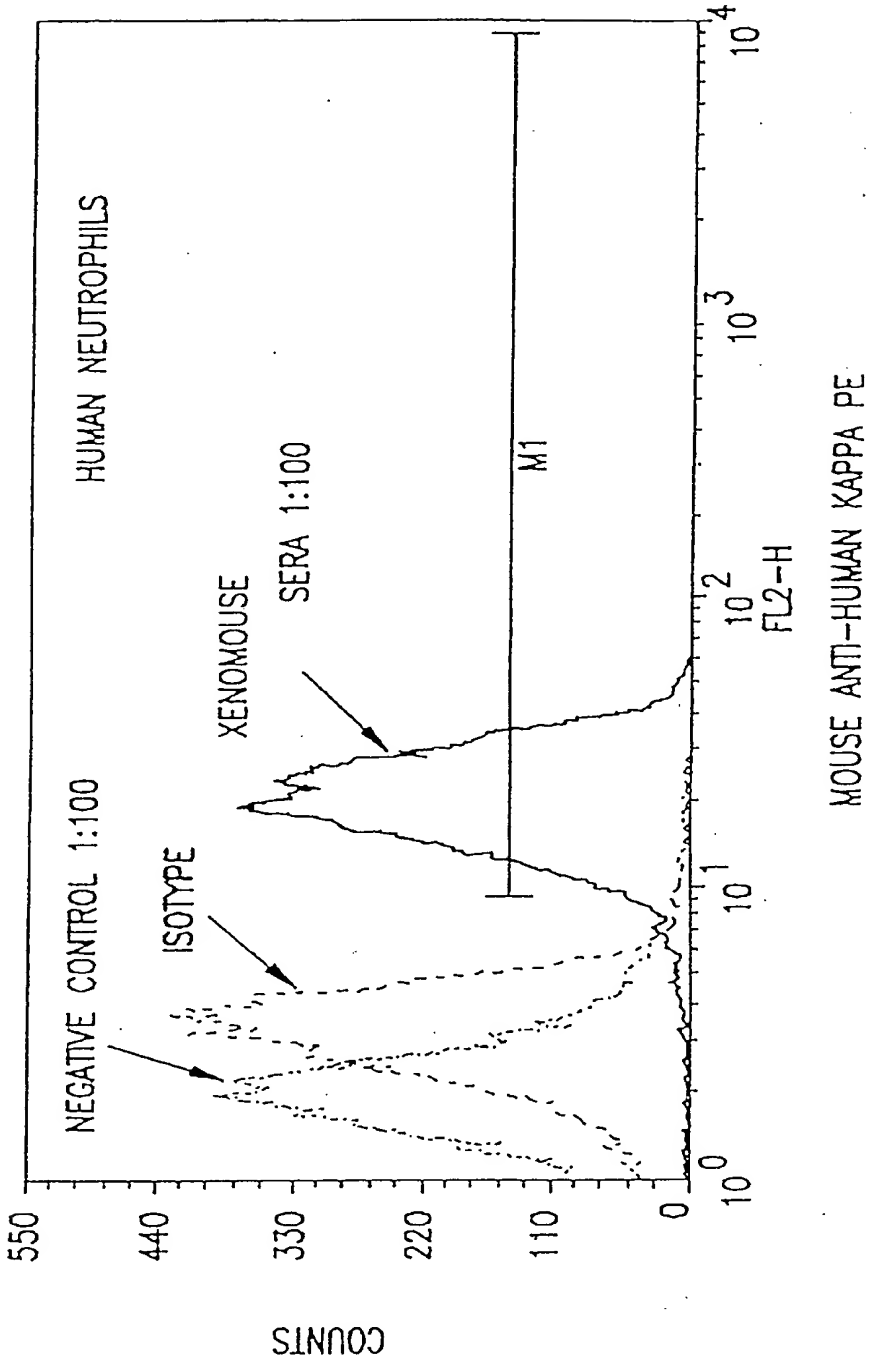

HUMAN NEUTROPHILS
NEGATIVE CONTROL 1:100
ISOTYPE
XENOMOUSE
SERA 1:100
M1
COUNTS
550
440
330
220
110
0
10^0
10^1
10^2
10^3
10^4
FL2-H
MOUSE ANTI-HUMAN KAPPA PE

FIG.8

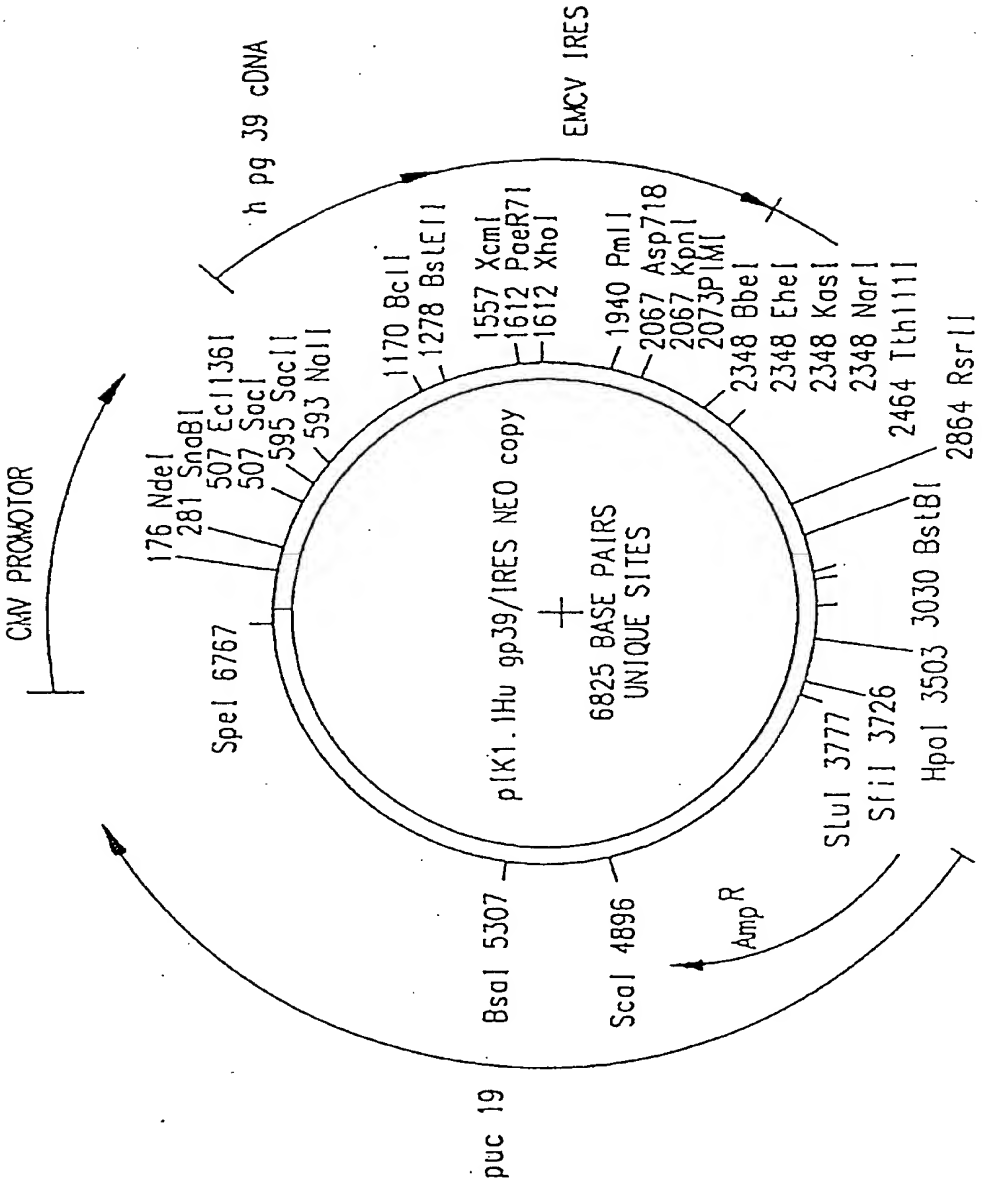

CMV PROMOTOR
h pg 39 cDNA
EMCV IRES
puc 19
Amp^R
pIK1.1Hu gp39/IRES NEO copy
6825 BASE PAIRS
UNIQUE SITES
176 NdeI
281 SnaBI
507 Ec11361
507 SacI
595 SacII
593 NaII
1170 BclI
1278 BsleII
1557 XcmI
1612 PaeR71
1612 XhoI
1940 PmlI
2067 Asp718
2067 KpnI
2073P1MI
2348 BbeI
2348 EheI
2348 KasI
2348 NarI
2464 Tth111I
2864 RsrII
3030 BstBI
3503 HpoI
3726 SfiI
3777 SluI
4896 ScaI
5307 BsaI
6767 SpeI

FIG.9

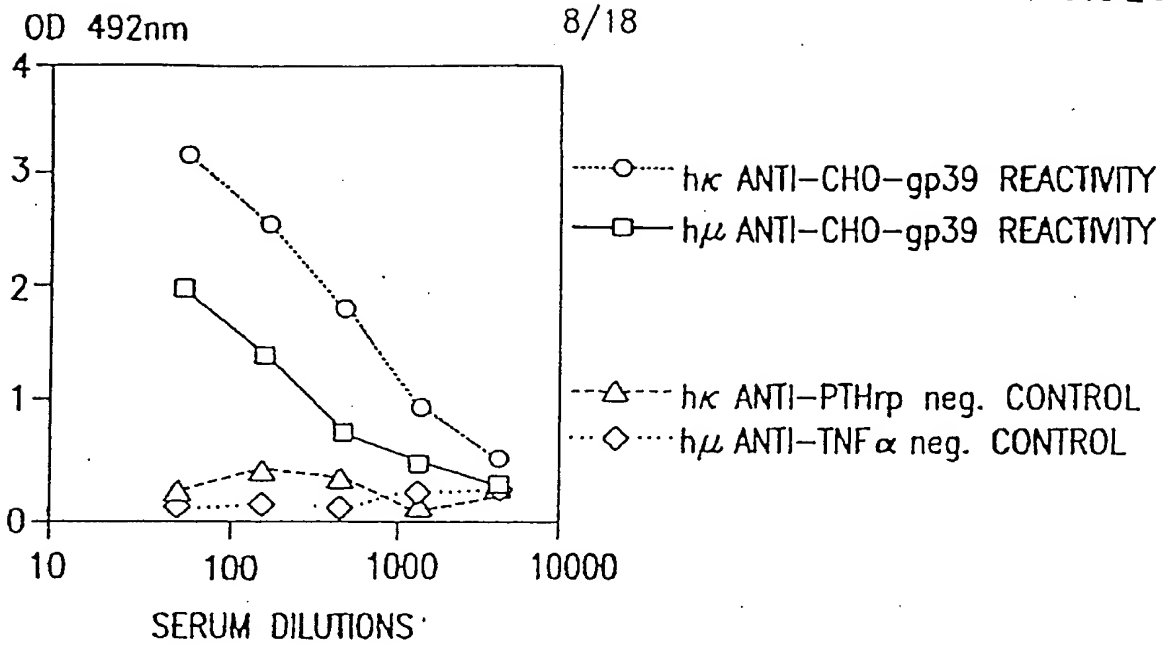

OD 492nm
4
3
2
1
0
10
100
1000
10000
SERUM DILUTIONS
hκ ANTI-CHO-gp39 REACTIVITY
hμ ANTI-CHO-gp39 REACTIVITY
hκ ANTI-PTHrp neg. CONTROL
hμ ANTI-TNFα neg. CONTROL

FIG.10

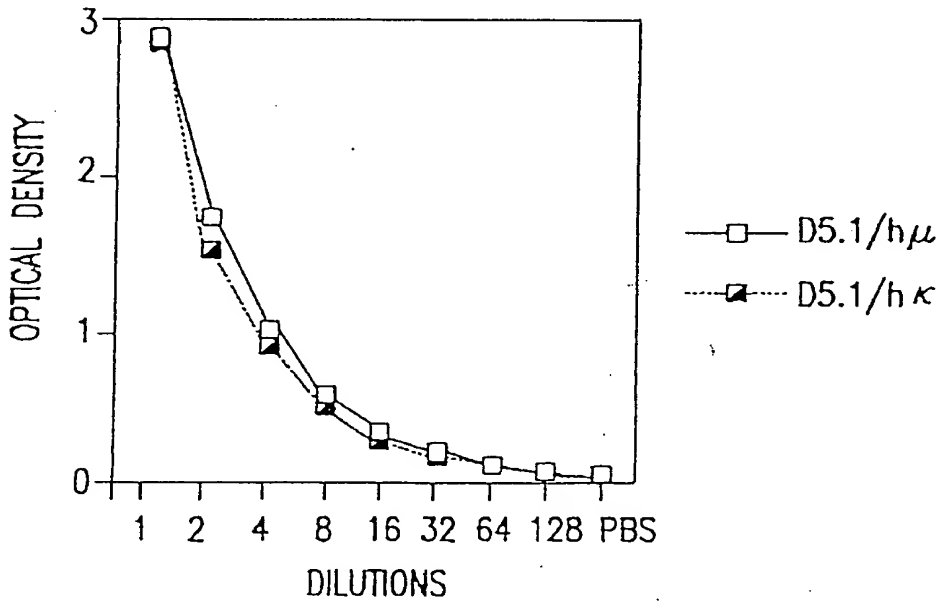

OPTICAL DENSITY
3
2
1
0
1
2
4
8
16
32
64
128
PBS
DILUTIONS
D5.1/hμ
D5.1/hκ

FIG.11

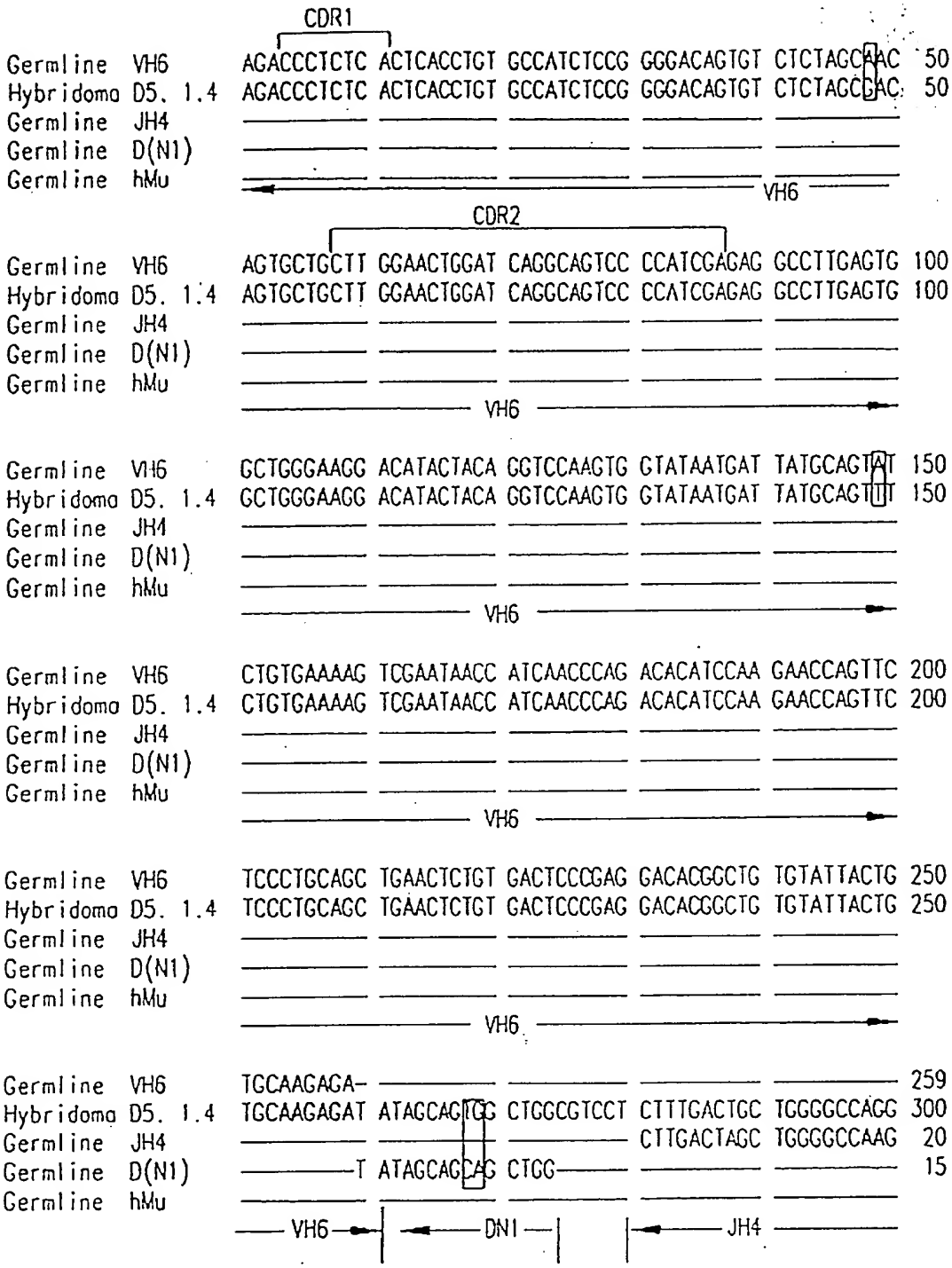

CDR1
Germline VH6 AGACCCTCTC ACTCACCTGT GCCATCTCCG GGGACAGTGT CTCTAGCAAC 50
Hybridoma D5. 1.4 AGACCCTCTC ACTCACCTGT GCCATCTCCG GGGACAGTGT CTCTAGCGAC 50
Germline JH4
Germline D(N1)
Germline hMu
VH6
CDR2
Germline VH6 AGTGCTGCTT GGAACTGGAT CAGGCAGTCC CCATCGAGAG GCCTTGAGTG 100
Hybridoma D5. 1.4 AGTGCTGCTT GGAACTGGAT CAGGCAGTCC CCATCGAGAG GCCTTGAGTG 100
Germline JH4
Germline D(N1)
Germline hMu
VH6
Germline VH6 GCTGGGAAGG ACATACTACA GGTCCAAGTG GTATAATGAT TATGCAGTAT 150
Hybridoma D5. 1.4 GCTGGGAAGG ACATACTACA GGTCCAAGTG GTATAATGAT TATGCAGTTT 150
Germline JH4
Germline D(N1)
Germline hMu
VH6
Germline VH6 CTGTGAAAAG TCGAATAACC ATCAACCCAG ACACATCCAA GAACCAGTTC 200
Hybridoma D5. 1.4 CTGTGAAAAG TCGAATAACC ATCAACCCAG ACACATCCAA GAACCAGTTC 200
Germline JH4
Germline D(N1)
Germline hMu
VH6
Germline VH6 TCCCTGCAGC TGAACTCTGT GACTCCCGAG GACACGGCTG TGTATTACTG 250
Hybridoma D5. 1.4 TCCCTGCAGC TGAACTCTGT GACTCCCGAG GACACGGCTG TGTATTACTG 250
Germline JH4
Germline D(N1)
Germline hMu
VH6
Germline VH6 TGCAAGAGA- 259
Hybridoma D5. 1.4 TGCAAGAGAT ATAGCAGCTGG CTGGCGTCCT CTTTGACTGC TGGGGCCAGG 300
Germline JH4 CTTGACTAGC TGGGGCCAAG 20
Germline D(N1) T ATAGCAGCAG CTGG 15
Germline hMu
VH6
DN1
JH4

FIG.12A

```
Germline  VH6          ———————— ———————— ———————— ———————— ———————— 259
Hybridoma D5. 1.4      GAACCCTGGT CACCGTCTCC TCAGGGAGTG CATCCGCCCC AACCCTTTTC 350
Germline  JH4          GAACCCTGGT CACCGTCTCC TCA———— ———————— ———————— 43
Germline  D(N1)        ———————— ———————— ———————— ———————— ———————— 15
Germline  hMu          ———————— ———————— ——GGGAGTG CATCCGCCCC AACCCTTTTC 27
                       ———— JH4 ————►|◄———— hμ ————
```

```
Germline  VH6          ———————— ———————— ———————— ———————— ———————— 259
Hybridoma D5. 1.4      CCCCTCGTCT CCTGTGAGAA TTCCCCGTCG GATACGAGCA GCGTGGCCGT 400
Germline  JH4          ———————— ———————— ———————— ———————— ———————— 43
Germline  D(N1)        ———————— ———————— ———————— ———————— ———————— 15
Germline  hMu          CCCCTCGTCT CCTGTGAGAA TTCCCCGTCG GATACGAGCA GCGTGGCCGT 77
                       ———————————————————— hμ ————————————————————►
```

FIG.12B

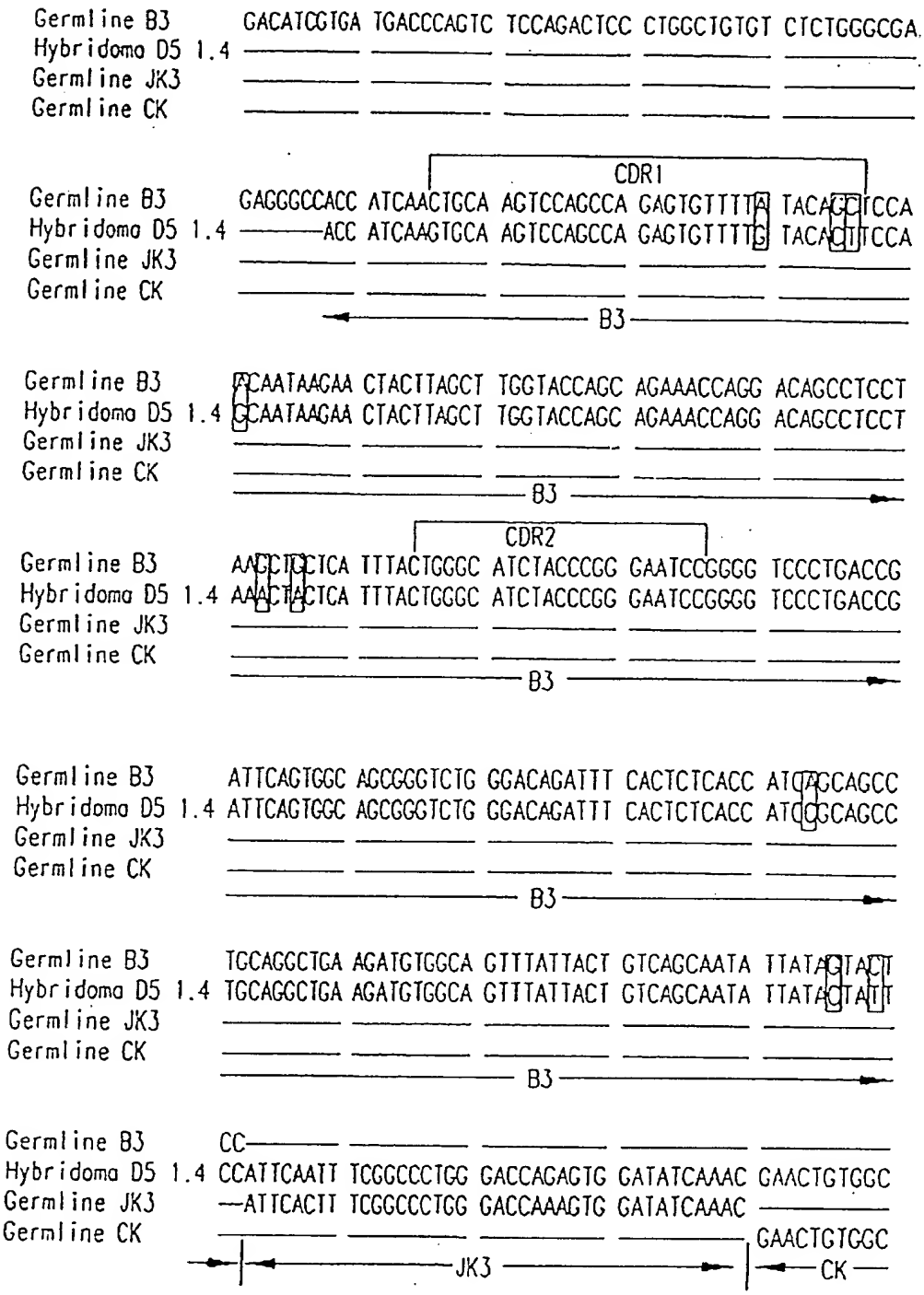

Germline B3 GACATCGTGA TGACCCAGTC TCCAGACTCC CTGGCTGTGT CTCTGGGCGA
Hybridoma D5 1.4
Germline JK3
Germline CK
CDR1
Germline B3 GAGGGCCACC ATCAACTGCA AGTCCAGCCA GAGTGTTTTA TACAGCTCCA
Hybridoma D5 1.4 ACC ATCAAGTGCA AGTCCAGCCA GAGTGTTTTG TACACTTCCA
B3
Germline B3 ACAATAAGAA CTACTTAGCT TGGTACCAGC AGAAACCAGG ACAGCCTCCT
Hybridoma D5 1.4 GCAATAAGAA CTACTTAGCT TGGTACCAGC AGAAACCAGG ACAGCCTCCT
CDR2
Germline B3 AAGCTGCTCA TTTACTGGGC ATCTACCCGG GAATCCGGGG TCCCTGACCG
Hybridoma D5 1.4 AAACTACTCA TTTACTGGGC ATCTACCCGG GAATCCGGGG TCCCTGACCG
Germline B3 ATTCAGTGGC AGCGGGTCTG GGACAGATTT CACTCTCACC ATCAGCAGCC
Hybridoma D5 1.4 ATTCAGTGGC AGCGGGTCTG GGACAGATTT CACTCTCACC ATCGGCAGCC
Germline B3 TGCAGGCTGA AGATGTGGCA GTTTATTACT GTCAGCAATA TTATAGTACT
Hybridoma D5 1.4 TGCAGGCTGA AGATGTGGCA GTTTATTACT GTCAGCAATA TTATACTATT
Germline B3 CC
Hybridoma D5 1.4 CCATTCAATT TCGGCCCTGG GACCAGAGTG GATATCAAAC GAACTGTGGC
Germline JK3 ATTCACTT TCGGCCCTGG GACCAAAGTG GATATCAAAC
Germline CK GAACTGTGGC
JK3
CK

FIG.13A

```
Germline B3                  ---------- ---------- ---------- ---------- ----------
Hybridoma D5 1.4 TGCACCATCT GTCTTCATCT TCCCGCCATC TGATGAGCAG TTGAAATCTG
Germline JK3                 ---------- ---------- ---------- ---------- ----------
Germline CK      TGCACCATCT GTCTTCATCT TCCCGCCATC TGATGAGCAG TTGAAATCTG
                 ---------- ---------- ---------- ---------- ---------- CK ---------->

Germline B3                  ---------- ---------- ---------- ---------- ----------
Hybridoma D5 1.4 GAACTGCCTC TGTTGTGTGC CTGCTGAATA ACTTCTATCC CAGAGAGGCC
Germline JK3                 ---------- ---------- ---------- ---------- ----------
Germline CK      GAACTGCCTC TGTTGTGTGC CTGCTGAATA ACTTCTATCC CAGAGAGGCC
                 ---------- ---------- ---------- ---------- ---------- CK ---------->

Germline B3                  ---------- ---------- ---------- ---------- ----------
Hybridoma D5 1.4 AAAGTACAGT GGAAGGTGGA TAACGCCCTC CAATCGGGTT GGGGAAAAA
Germline JK3                 ---------- ---------- ---------- ---------- ----------
Germline CK      AAAGTACAGT GGAAGGTGGA TAACGCCCTC CAATCGGGT- ----------
                 ---------- ---------- ---------- ---------- ---------- CK ---------->
```

FIG.13B

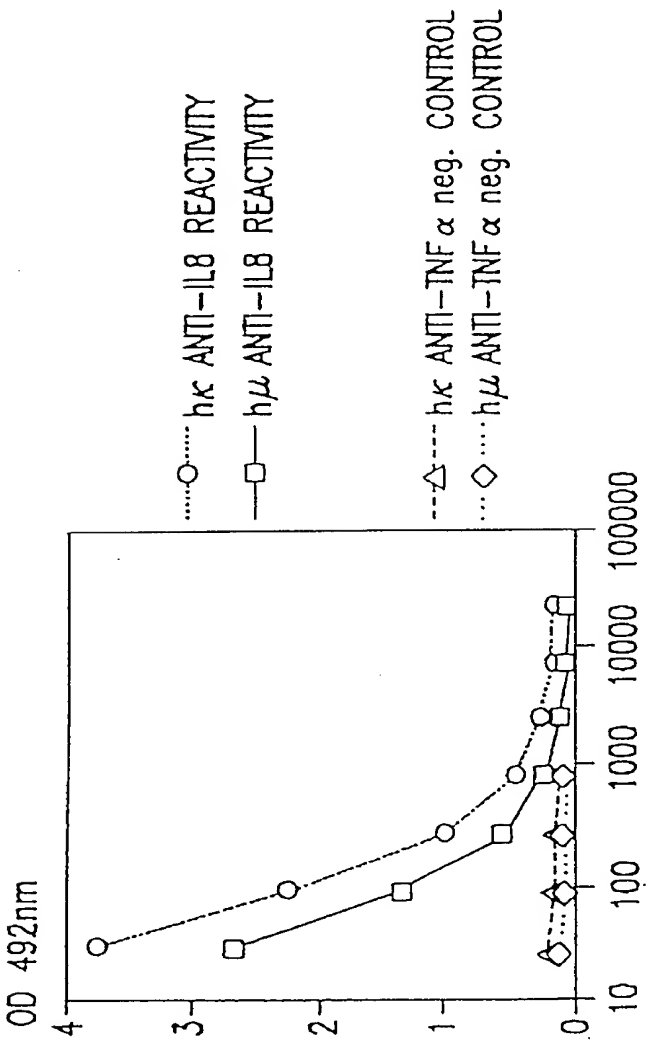

hκ ANTI-IL8 REACTIVITY
hμ ANTI-IL8 REACTIVITY
hκ ANTI-TNFα neg. CONTROL
hμ ANTI-TNFα neg. CONTROL
OD 492nm
4
3
2
1
0
10
100
1000
10000
100000
SERUM DILUTIONS

FIG.14

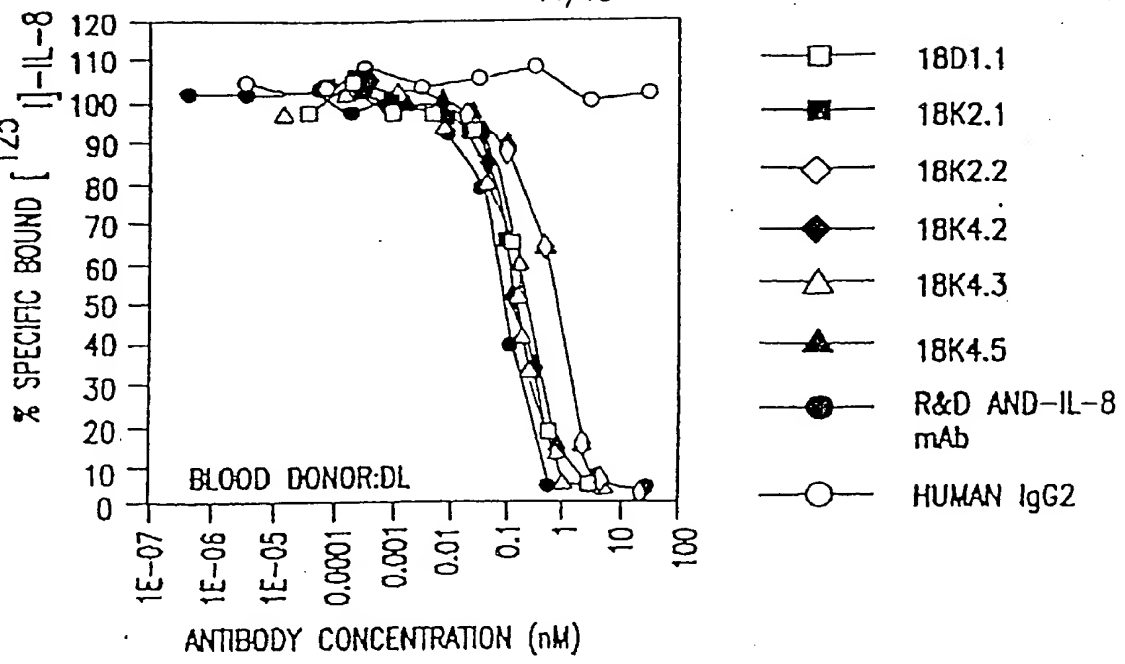


FIG.15A

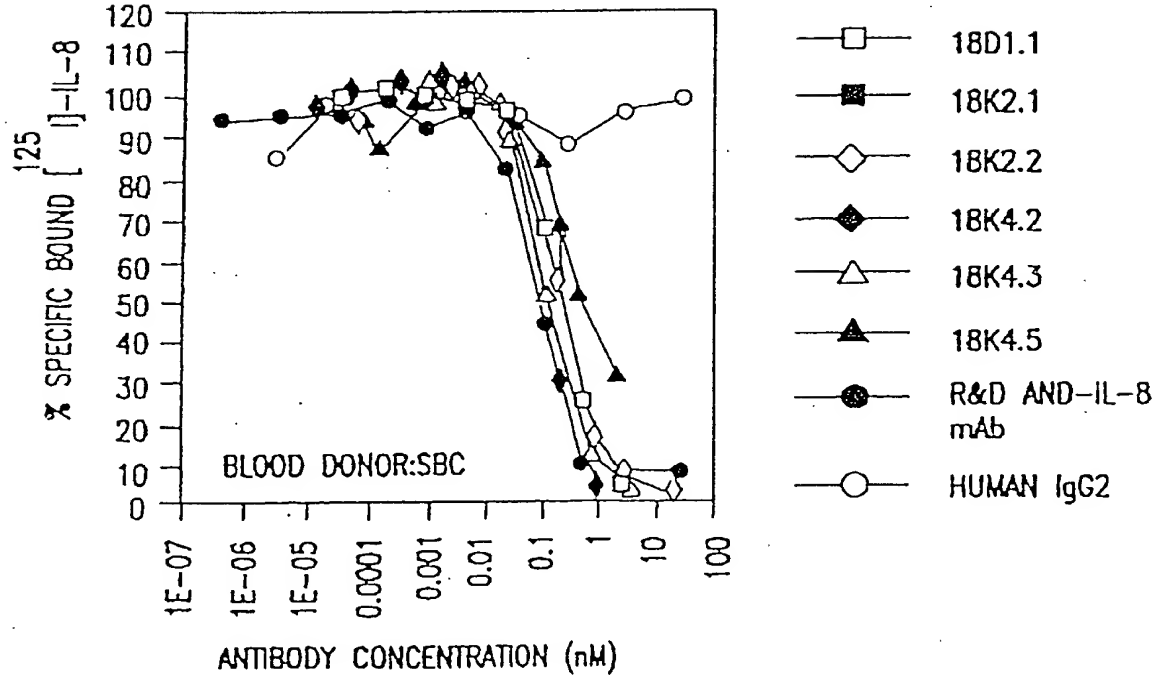


FIG.15B

[CCTGTCCCTCACCTGCGCTGTCTATGGTGGGTCCTTCAGTGGTTACTACTGGAGCTGGATCCGCC

AGCCCCCAGGGAAGGGACTGGAGTGGATTGGGGAAATCAATCAAAGTGGAAGCACCAATTACAA

CCCGTCCCTCAAGAGTCGAGTCATCATATCAATAGACACGTCCAAGACCCAGTTCTCCCTGAAGT

TGAGCTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGAGA][GACTCCCC][ATGCT

TTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCAG]CCTCCACCAAGGGCCCATCGG

TCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGC(GC)GCCCTGGGCTGCCTG

GTCAAGGACTACTTCC

FIG. 16A

[CAGTCTCCATCCTCCCTGTCTGCATCTGTAGGCGACAGAGTCACCATCACTTGCCAGGCGAGTC

AGGACATTAGTAAGTTTTTAAGTTGGTTTCAACAGAAACCAGGGAAAGCCCCTAAACTCCTGATC

TACGGTACATCCTATTTGGAAACCGGGGTCCCATCAAGTTTCAGTGGAAGTGGATCTGGGACAGA

TTTTACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGTTGCAACATATTTCTGTAACAGNATG

ATGATCTCCC][ATACACTTTCGGCCCTGGGACCAAAGTGGATATCAAAC]GAACTGTGGCTGCAC

CATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCC

TGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC

FIG. 16B

[AGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGGCATGCACTGGNT

CCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGAAATATCATATGATGGAAGTAATAAA

TACTATGTAGACTCCGTGAAGGGCCGACTCACCATCTCCAGAGACAATTCCAAGAACACGCTGT

ATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA][CCGAC

TGGGGAT][CTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAG]CCTCCACCAAGG

GCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGC(GC)GGCCCT

GGGCTGCCTGGTCCAAGGACTACTTCCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCTC

TGACCAG

FIG. 16C

[CTGACNCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATCAACTGCAAGT

CCAGCCAGAGTGTTTTATACATCTCCAACAATAAAACTACTTAGCTTGGTACCAGCAGAAACCA

GGACAGTCTCCTAAACTGCTCATTTACTGGGCATCTACCCGGAAATCCGGGGTCCCTGACCGATT

CAGTGGCAGCGGGTCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTG

GCAGTTTATTACTGTCAACAGTATTATGATACTCC][ATTCACTTTCGGCCCTGGGACCAAAGTGG

ATATCAAAC]GAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAA

TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTG

GAAGGTGGNTAACGCCCCA

FIG. 16D

[TCCCTCACCTGCGCTGTCTATGGTGGGTCCTTCAGTGGTTACTACTGGACCTGGATCCGCCAGCC
CCCAGGGAAGGGGCTGGAGTGGATTGGGGAAATCATTCATCATGGAAACACCAACTACAACCCG
TCCCTCAAGAGTCGAGTCTCCATATCAGTTGACACGTCCAAGAACCAGTTCTCCCTGACACTGAG
CTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGAGG][GGGAGCAGTGGCTGCG][T
TTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAG]CCTCCACCAAGGGCCCATCGGT
CTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGC(GC)GGCCCTGGGCTGCCTG
GTCAAGGACTACTTCCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCTCTGACCAGCGGC
GTGCACACCTTCCCA

FIG. 16E

[TGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCAGGC
GAGTCAGGACATTAGTAACTATTTAAATTGGTATCAACAGAAAGCAGGGAAAGCCCCTAAGGTCC
TGATCTACGCTGCATCCAATTTGGAAGCAGGGGTCCCATCAAGGTTCAGTGGAAGTGGATCTGGG
ACAGATTTTACTTTCACCATCAGCAGCCTGCAGCCTGAAGATATTGCAACATATTATTGTCAACA
CTATGATAATCT]A[CTCACTTTCGGCGGAGGGACCAAGGTAGAGATCAAAC]GAACTGTGGCTGC
ACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGACTGCCTCTGTTGTGTG
CCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGG

FIG. 16F

AGTCTCTGAAGATCTCCTGTAAGGGTTCTGGATACAGCTTTACCAGCTACTGGATCGGCTGGGTG

CGCCAGATGCCCGGGAAAGGCCTGGAGTGGATGGGGATCATCTATCCTGGTGACTCTGATACCA

GATACAGCCCGTCCTTCCAAGGCCAGGTCACCATCTCAGCCGACAAGTCCATCAGCACCGCCTA

CCTGCAGTGGAGCAGCCTGAAGGCCTCGGACACCGCCATGTATTACTGTGCGAGACA][GGACGG

TG][ACTCCTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAG]CCTCCACCAAGGG

CCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGC(GC)GGCCCTG

GGCTGCCTGGTCCAAGGACTACTTCCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCT

GACCAGCGGCGTGCACACCTTCCCACTGCCA

FIG. 16G

TGTCTGCATCTATTGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAACTA

TTTAAATTGGTATCAGCAGAAACCAGGGCAAAGCCCCTAAGTTCCTGATCTATGGTGCATCCAGT

TTGGAAAGTGGGGTCCCATCANGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCAT

CAGCAGCCTGCAACCTGNGGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTAACCC]T[CTC

ACTTTCGGCGGNGGGACCAANGTGGAGATCAAAC]GAACTGTGGCTGCACCATCTGTCTTCATCT

TCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCT

ATCCCAGAGAGGCCAAAGTACA

FIG. 16H